Use of Mesenchymal Stem Cells in COVID-19

Özgür Doğuş Erol[1,2], Fatima Aerts-Kaya[1,2*]

1. Hacettepe University Graduate School of Health Sciences, Department of Stem Cell Sciences, Ankara, Turkey
2. Hacettepe University Center for Stem Cell Research and Development, Ankara, Turkey

***Corresponding author:**

Fatima Aerts-Kaya MD PhD

Hacettepe University

Center for Stem Cell Research and Development

06100 Sıhhiye, Ankara, Turkey

Email: fatimaaerts@yahoo.com

Tel: +90-312-3052821

Abstract word count: 174

Manuscript word count: 1425

Abstract

Since the start of the coronavirus SARS-CoV2 (COVID-19) outbreak in China, in the end of 2019, the virus has rapidly spread throughout the World, causing a path of destruction behind it, with mortality figures exceeding 7.000.000 globally, and a death toll reaching numbers of over 400.000. Economically, the pandemic has been estimated to cause a World-wide crise similar or worse than World War II. While physians are just barely able to cope with the amount of patients, lack of knowledge on the pathogenesis of the disease, its targets and possible effective treatments are major hurdles in the design of proper treatment strategies. Current treatment options using a variety of anti-viral, anti-bacterial and other drugs, have shown to be often insufficient and in severe cases of COVID-19 disease, the use of stem cells, and in particular mesenchymal stem cells, has been proposed as auxiliary treatment, based on their proven capacity for potent immunomodulation and tissue regeneration. Here, we provide a brief overview of current data on the use of MSCs for treatment of COVID-19.

SARS-CoV2 and COVID-19

Since the start of the coronavirus SARS-CoV2 (COVID-19) outbreak in China, 2019, the virus has shown an unprecedented and previously unseen rapid global spread, and was pronounced a pandemic by the WHO, just three months after the first reports of a cluster of unexplained pneumonia's in Wuhan, Hubei province (https://www.who.int/news-room/detail/27-04-2020-who-timeline---covid-19). As of today, the disease has spread to over 7 million confirmed cases worldwide and resulted in over 400.000 deaths (https://www.worldometers.info/coronavirus). In severe cases, the disease is initially characterized by fever, cough, dyspnea and lymphopenia, followed by progressive signs of pneumonia, causing severe acute respiratory dyspnea syndrome (ARDS), deteriorating multi-organ dysfunction (MOD) and coagulopathy, which all together may result in death. Thus far, no single treatment (drug or vaccine) has been

identified with the potential to cure or prevent disease in patients. However, since overactivation of the immune system and systemic inflammation are considered main factors in COVID-19 pathogenesis, attempts to dampen the overactive immune system has been the target of many studies.

Mesenchymal stem cells

Mesenchymal stem cells (MSCs) are multipotent stromal tissue-derived progenitor cells that have the capability to differentiate into many different lineages, including osteogenic, chondrogenic, adipogenic and myogenic direction. These cells have been extensively studied in clinical trials and treatment strategies for regenerative purposes. In addition, these cells also display an important immunomodulatory capacity and have been shown to be able to suppress or regulate the overactivated immune system in the treatment of autoimmune diseases and graft-versus-host disease. In the past, the effects of MSC transplantation for treatment of acute lung diseases, pneumonia, ARDS, inflammatory and infectious lung diseases have been assessed in both preclinical animal models and clinical trials (reviewed by [1, 2]). These studies have shown that in general, use of MSCs appears to be well tolerated, safe and absent of severe side effects. In addition, MSCs were shown to have a positive effect on pulmonary fibrosis through inhibition of lung damage, reduced inflammation, normalized immune responses and even through promotion of alveolar fluid clearance. Furthermore, it was also shown that MSCs were able to produce both anti-microbial and pain-relieving molecules. Of note, many studies have shown that upon systemic infusion MSCs rapidly biodistribute and initially accumulate in the lungs, before moving on to different locations. This may prove to be a great advantage for the treatment of pulmonary disease with MSCs, especially since it has been shown that while lodged in the lungs, MSCs may release a wide variety of anti-inflammatory cytokines, anti-microbial peptides, angiogenic growth factors, and extracellular vesicles [3].

Rationale for use of MSCs for treatment of COVID-19 related disease

Understandingly, the interest in the use of MSCs for the treatment of COVID-19-related pneumonia, ARDS and pulmonary fibrosis has increased rapidly and since the start of the outbreak, 30 new clinical trials were registered on https://clinicaltrials.gov using MSCs for the treatment of COVID-19 (data per 30 per June 9th 2020, Table 1). In 50% (n=15) of these clinical trials, researchers intend to use umbilical cord (UC, Wharton Jelly) as the source of MSCs, whereas in 7 studies use of BM-MSCs is planned. The preference of most researchers for UC-MSCs is based on the facts that UC-MSCs can be easily isolated from the umbilical cord without the need for invasive procedures and that their use is not restricted by ethical issues. Furthermore, they can be rapidly expanded to numbers sufficient for transplantation. Similar to BM-derived MSCs, these cells are immunologically inert, since they express MHC class I molecules, but lack expression of MHC class II antigens on their surface, but in contrast to BM-MSCs, UC-MSCs have been shown to have a broader plasticity and lack tumorigenicity. Most importantly however, UC-MSCs can be easily isolated, expanded and cryopreserved, while quality control is performed, allowing the manufacturing of off-the-shelf-products that can be ready for use whenever needed. This brings a great advantage compared to the use of autologous BM or adipose-tissue derived stem cells (ASCs/ADSCs and stromal vascular fraction [SVF] cells), which require at least 1-2 weeks of expansion, a period that may be too long for critically ill COVID-19 patients.

When Pubmed (https://pubmed.ncbi.nlm.nih.gov) was searched for “COVID-19 and MSCs” 19 manuscripts were found. Using additional searches for “stem cells and COVID-19” resulted in the collection of in total 24 manuscripts (Figure 1). Whereas most manuscripts were reviews discussing the possible use and safety issues of MSCs for the treatment of COVID-19 related diseases, few actual data on the effects of MSCs on COVID-19 are available.

Table 1. Clinical trials for treatment of COVID-19 with MSCs

Nr	Clinical trial identifier	Status	Title	Biological	Country
1	NCT04416139	Recruiting	MSC for ARDS Due for COVID-19	UC-MSCs	Mexico
2	NCT04400032	Not yet recruiting	Cellular Immuno-Therapy for COVID-19 ARDS	MSCs	Canada
3	NCT04399889	Not yet recruiting	hCT-MSCs for COVID-19 ARDS	Human cord tissue MSCs	
4	NCT04398303	Not yet recruiting	ACT-20 in Patients With Severe COVID-19 Pneumonia	UC-MSCs (ACT-20-MSC)	
5	NCT04397796	Not yet recruiting	Safety of Therapeutic Tx With Immuno-modulatory MSC in Adults With COVID-19 Infection Requiring Mechanical Ventilation	Allogeneic BM-MSCs	
6	NCT04397471	Not yet recruiting	A Study to Collect Bone Marrow for Process Development and Production of BM-MSC to Treat Severe COVID19 Pneumonitis	Allogeneic BM-MSCs	United Kingdom
7	NCT04392778	Recruiting	Clinical Use of Stem Cells for the Treatment of COVID-19	UC-MSC	Turkey
8	NCT04390152	Not yet recruiting	Safety and Efficacy of Intravenous Wharton's Jelly Derived MSCs in ARDS Due to COVID-19	WJ-MSC	Colombia
9	NCT04390139	Recruiting	Evaluation of MSCs for the Treatment of Patients With ARDS due to COVID-19	WJ-MSC (XCEL-UMC-BETA)	Spain
10	NCT04382547	Enrolling by invitation	Treatment of COVID-19 Associated Pneumonia With Allogenic Pooled Olfactory Mucosa-derived MSCs	Allogenic olfactory mucosa-derived MSCs	Belarus
11	NCT04377334	Not yet recruiting	MSCs in Inflammation-Resolution Programs of COVID-19 Induced ARDS	Allogeneic BM-MSCs	Germany
12	NCT04371601	Active, not recruiting	Safety and Effectiveness of MSCs in the Treatment of Pneumonia of COVID-19	UC-MSCs	China
13	NCT04371393	Recruiting	MSCs in COVID-19 ARDS	Allogeneic BM-MSCs (Remestemcel-L)	USA
14	NCT04366830	No longer available	Intermediate-size Expanded Access Program (EAP), MSC for ARDS Due to COVID-19 Infection	Allogeneic BM-MSCs (Remestemcel-L)	USA
15	NCT04366063	Recruiting	MSC Therapy for SARS-CoV2-related ARDS	MSCs unspecified	Iran
16	NCT04361942	Recruiting	Treatment of Severe COVID-19 Pneumonia With Allogeneic MSCs (COVID-MSV)	Allogeneic MSCs from unspecified source	Spain
17	NCT04355728	Recruiting	Use of UC-MSCs for COVID-19 Patients	UC-MSCs	USA
18	NCT04352803	Not yet recruiting	Adipose MSCs for Abatement of SARS-CoV2 Respiratory Compromise in COVID-19	Autologous Adipose MSCs	
19	NCT04346368	Not yet recruiting	BM-Derived MSC Treatment for Severe Patients With COVID-19	BM-MSCs	China
20	NCT04345601	Not yet recruiting	MSCs for the Treatment of SARS-CoV2 Induced Acute Respiratory Failure (COVID-19 Disease)	Allogeneic BM-MSCs	USA
21	NCT04339660	Recruiting	Clinical Research of Human MSC in the Treatment of COVID-19 Pneumonia	UC-MSCs	China
22	NCT04333368	Recruiting	Cell Therapy Using Umbilical Cord-derived MSCs in SARS-CoV2-related ARDS	UC-MSCs	France
23	NCT04313322	Recruiting	Treatment of COVID-19 Patients Using Wharton's Jelly-MSCs	WJ-MSCs	Jordan
24	NCT04293692	Withdrawn	Therapy for Pneumonia Patients Infected by 2019 Novel Coronavirus	UC-MSCs	China
25	NCT04288102	Recruiting	Treatment With MSCs for Severe COVID-19	MSCs unspecified	China
26	NCT04276987	Not yet recruiting	Clinical Study on Inhalation of MSCs Exosomes Treating Severe Novel Coronavirus Pneumonia	MSC-derived exosomes	
27	NCT04273646	Not yet recruiting	Study of Human UC-MSCs in the Treatment of Severe COVID-19	UC-MSCs	China
28	NCT04269525	Recruiting	UC-Derived MSCs Treatment for the 2019-novel Coronavirus(nCOV) Pneumonia	UC-MSCs	China
29	NCT04252118	Recruiting	MSC Treatment for Pneumonia Patients Infected With COVID-19	MSCs unspecified	China
30	NCT03042143	Recruiting	Repair of ARDS by Stromal Cell Administration (REALIST) (COVID-19)	Human CD362 enriched UC-MSCs	United Kingdom

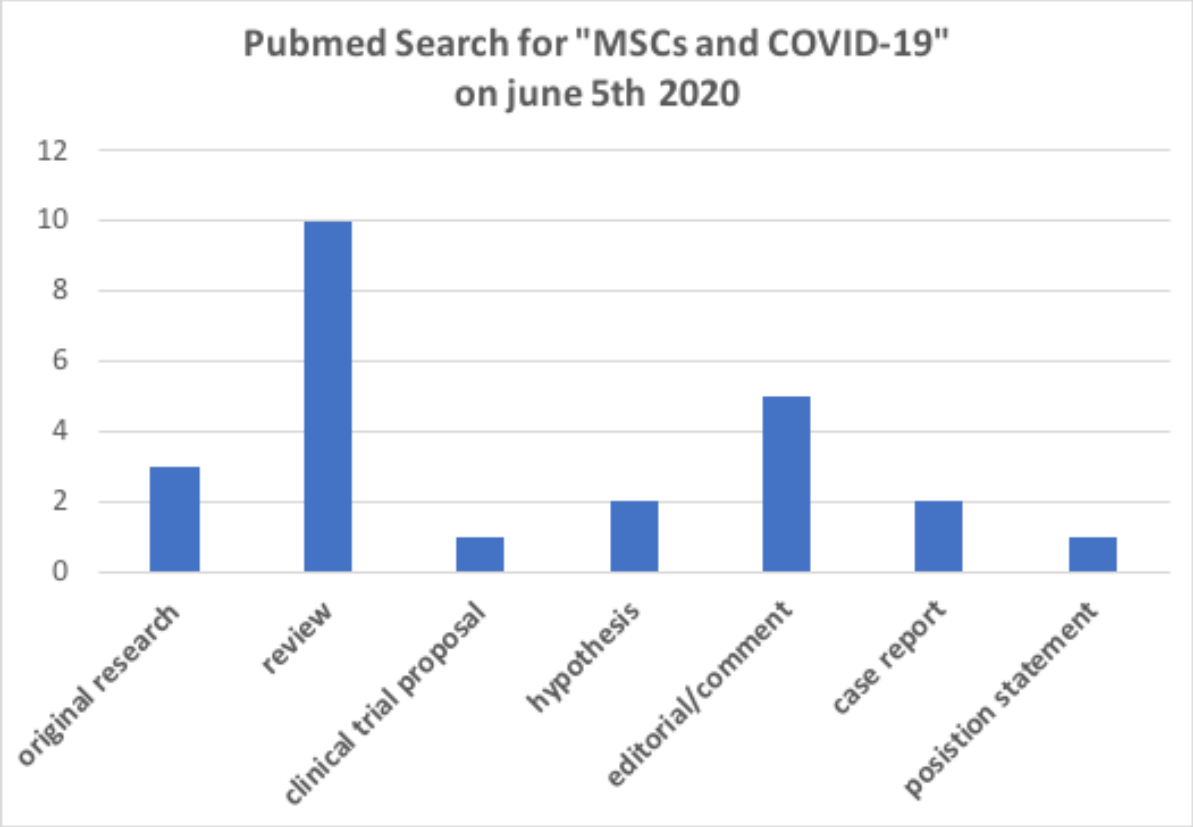

Figure 1. Pubmed search for papers on COVID-19 and MSCs

Current status of use of MSCs for treatment of COVID-19 disease

The aim of the first published clinical study using MSCs for treatment of COVID-19 was to prevent, decrease or reverse the cytokine storm associated with COVID-19 [4], whereas the second study and the case reports retrospectively assessed the effects of compassionate use of MSCs for COVID-19 related multiorgan failure [5, 6]. Data from the original manuscripts and case reports extracted from the Pubmed search are provided in Table 2.

Leng et al. infused $1x10^6$ MSCs intravenously into 7 patients with COVID-19 pneumonia and compared the effects with the placebo group [4]. Patients treated with MSCs showed a remarkable recovery and a significant decrease in the serum levels of the pro-inflammatory cytokine TNF-α, and a simultaneous increase in levels of anti-inflammatory IL-10.

Table 2. Overview of studies using MSCs for treatment of COVID-19 related disease

Patient characteristics (N, age)	Dose, ROA, source, f-up, diagnosis	Aim	Effect	Side Effect	Clinical outcome	Ref
MSC Tx group Critically severe (n=1, age 65), Severe (n=4, age 45-65), Common (n=2, age 51-57) *Control group* Severe (n=3, age 46-75)	$1x10^6$ MSCs/kg, i.v., MSC source unspecified, f-up 14 days, RT-PCR Placebo, f-up 14 days, RT-PCR	To decrease, prevent or reverse CK storm Control group	Pulmonary function ↑, PBL ↑, CRP ↓, IL-10 ↑, TNFα ↓	No acute or allergic reactions, delayed hyper-sensitivity or secondary infections	Recovery (n=4) Discharged (n=3) Death (n=1) ARDS (n=1) Stable (n=1)	[4]
MSC Tx group Severe (n=25, age median 70)	$1x10^6$ MSCs/kg once (n=7); twice (n=7); or thrice, 5 days apart (n=11); source unspecified, iv, f-up 72 hrs after infusion, RT-PCR	To retrospectively assess the therapeutic potential of MSCs for severe COVID-19	No effect on WBC counts, CRP, PCT and IL-6 levels; LAC, cTnT and CK-MB increased	liver dysfunction, heart failure and allergic rash (n=3)	Recovery (n=25)	[5]
MSC Tx Critically severe (n=1, age 65)	$5x10^7$ hUCMSCs thrice, 3 days apart, iv	Compassionate use for severe organ injury caused by inflammatory response	serum bilirubin, CRP, ALT/AST decreased, WBC decreased, PBL increased	No side effects observed		Liang et al. 2020, Chinax iv.org
MSC Tx Critically severe (n=1, age 54)	$1x10^6$ cells/kg hWJ-MSCs, iv, f-up 7 days, RT-PCR	Compassionate use for severe organ injury caused by inflammatory response	CRP decreased, IL-6 and TNFa decreased, PBL increased, inflammatory factors reduced	No acute infusion-related or allergic reactions, no delayed hypersensitivity or secondary infections	oxygen saturation >98%, recovery and discharge	[6]

MSC: Mesenchymal Stem Cells; Tx: transplantation; ROA: Route of administration; f-up: follow-up; RT-PCR: Real Time Polymerase Chain Reaction; CK: Cytokine; UC-MSCs: Umbilical Cord-derived MSCs; WJ-MSCs: Wharton Jelly MSCs; COVID-19: SARS-Cov2 related disease; CRP: C-reactive protein; PBL: Peripheral blood lymphocytes; ALT/AST: alanine aminotransferase/aspartate aminotransferase; ARDS: Acute Respiratory Distress Syndrome.

Importantly, RNASeq analysis of the transplanted MSCs revealed that the cells did not express the SARS-CoV receptor ACE2 or the serine protease TMPRSS2 [4]. Since it was recently shown that the SARS-CoV2 virus uses ACE2 for entry, and TMPRSS2 for S protein priming [7], these data may indicate that MSCs may be relatively resistant for COVID-19 infection. The researchers also found evidence that MSCs exert their immunomodulatory effects through expression of anti-inflammatory and trophic factors like TGF-β, HGF, LIF, GAL, NOA1, FGF, VEGF, EGF, BDNF, and NGF [4] and might therefore be able to prevent or dampen the

cytokine storm associated with COVID-19. Furthermore, mass cytometry (CyTOF) analysis of the patients' peripheral blood lymphocyte (PBL) subsets revealed that in the severe patients, but not in the mild patients, both the regulatory T cells and dendritic cell (DC) numbers increased after MSC infusion, overactivated CXCR3+ T cells and NK cells nearly disappeared and the numbers of the other cell subpopulations were restored to normal levels, including the regulatory DC population [4]. These first data of MSC infusion as auxiliary treatment in critically ill COVID-19 patients are certainly encouraging, although caution should be taken that data from a single clinical trial should not be generalized and require confirmation. However, many variables may influence the efficacy and effects of MSCs on COVID-19 patients, including route of administration (ROA: intravenous or intratracheal administration), dose, single or repetitive administration, severity of the COVID-19 disease and symptoms, timing, source of the cells (allogeneic/autologous; UC/WJ-MSC, ASC/ADSC/SVF or BM-MSC), culture conditions, rate of infusion, etc.

Data from the use of MSCs for the treatment of ARDS induced by other causes than COVID-19 and other causes of lung injury, such as bronchopulmonary dysplasia, acute lung injury (viral and bacterial infections, as well as toxic insults) and chronic injury, such as asthma, COPD, lung fibrosis are reviewed by Qu and Rogers and generally confirm safety, as well as efficacy with reduced mortality in the groups treated with systemic or intratracheal administration of MSCs [1, 8]. Working mechanisms of MSCs include not only mitigation of inflammation, but also direct inhibition of pulmonary fibrosis, lung tissue regeneration, anti-apoptotic effects on injured cells, alveolar fluid clearance and production of extracellular vesicles (summarized in Figure 2).

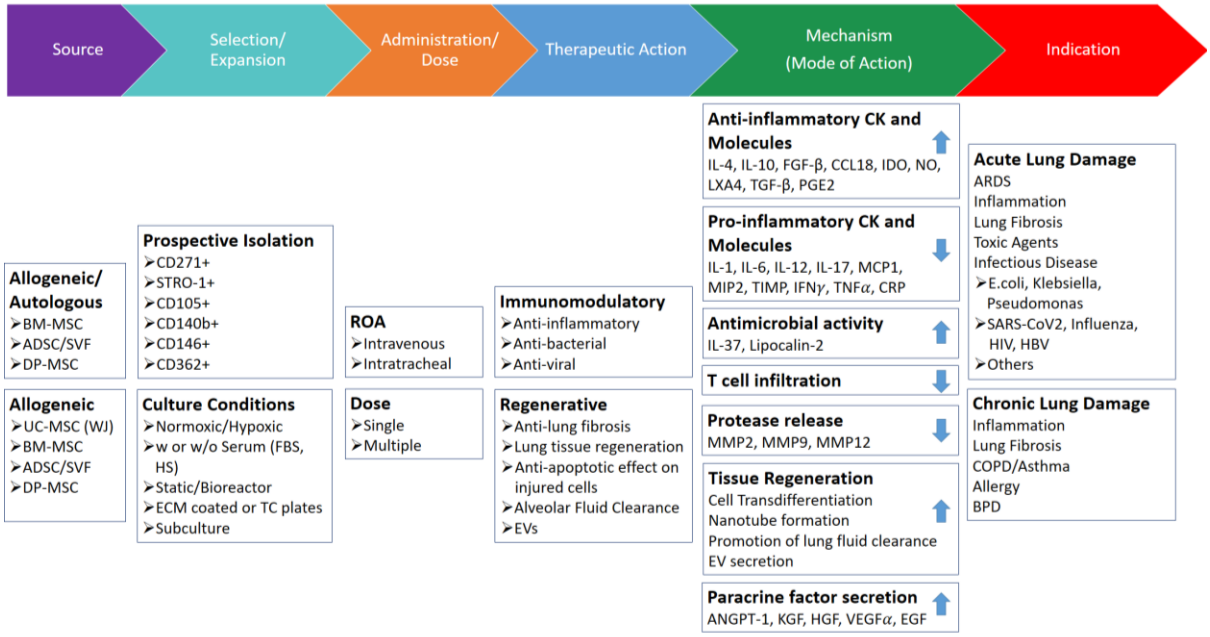

Figure 2. Use of MSCs for COVID-19 related disease and other causes of lung damage

MSCs: Mesenchymal Stem Cells; BM: Bone Marrow; ADSC: Adipose Derived Stem Cells; SVF: Stromal Vascular Fraction; DP: Dental Pulp; UC: Umbilical Cord; WJ: Wharton Jelly; FBS: Fetal Bovine Serum; HS: Human Serum; ECM: Extracellular Matrix; TC: Tissue Culture treated; EVs: Extracellular vesicles; CK: Cytokine; IL: Interleukin; FGF-β: Fibroblast Growth Factor beta; CCL: C-C Motif Chemokine Ligand, IDO: Indoleamine-pyrole 2,3-dioxygenase; NO: Nitric oxide; LXA4: Lipocalin A4; TGF-β: Tumor Growth Factor beta; PGE2: Prostaglandin E2; MCP-1: Monocyte Chemoattractant Protein 1; MIP: Macrophage Inflammatory Protein; TIMP: Tissue Inhibitors of Metalloproteinases; IFNγ: Interferon gamma; TNFα: Tumor Necrosis Factor alpha; CRP: C-reactive protein; MMP: Metalloproteinase; ANGPT-1: Angiopoietin-1; KGF: Keratinocyte Growth Factor; HGF: Hepatocyte Growth Factor; VEGFα: Vascular Endothelial Growth Factor alpha; EGF: Epithelial Growth Factor; ARDS: Acute Respiratory Distress Syndrome; HIV: Human Immunodeficiency Virus; HBV: Hepatitis B Virus; COPD: Chronic Obstructive Pulmonary Disease; BPD: Bronchopulmonary Disease.

Conclusions

The globally fast spreading COVID-19 disease is caused by the pandemic SARS-CoV2 and is characterized by rapidly progressing pneumonia, ARDS, multiorgan failure and coagulopathy which has been associated with an overactivated immune system trying to cope with the infection. As current treatment strategies are insufficient, stem cell therapies have proposed as additional treatment options. Experience with the use of MSCs for their immunomodulatory effects and regenerative capacities in the treatment of specifically pulmonary fibrosis and ARDS has shown the broad potential of these cells, a favorable safety profile and improvement of the disease. MSCs show therefore great promise as an optional treatment strategy for prevention of the cytokine storm, lung fibrosis and ARDS caused by COVID-19. Retrospective

analysis and initial data from compassionate use of MSCs for treatment of COVID-19 pneumonia and multiorgan failure show promising results. Data to be obtained from the many planned clinical trials using MSCs from different sources for the treatment of COVID-19 will be available in the next coming years and will reveal the full potential of these cells.

References

[1] C.J. Rogers, R.J. Harman, B.A. Bunnell, M.A. Schreiber, C. Xiang, F.S. Wang, A.F. Santidrian, B.R. Minev, Rationale for the clinical use of adipose-derived mesenchymal stem cells for COVID-19 patients, J Transl Med 18(1) (2020) 203.

[2] J. Behnke, S. Kremer, T. Shahzad, C.M. Chao, E. Bottcher-Friebertshauser, R.E. Morty, S. Bellusci, H. Ehrhardt, MSC Based Therapies-New Perspectives for the Injured Lung, J Clin Med 9(3) (2020).

[3] M. Khoury, J. Cuenca, F.F. Cruz, F.E. Figueroa, P.R.M. Rocco, D.J. Weiss, Current status of cell-based therapies for respiratory virus infections: applicability to COVID-19, Eur Respir J 55(6) (2020).

[4] Z. Leng, R. Zhu, W. Hou, Y. Feng, Y. Yang, Q. Han, G. Shan, F. Meng, D. Du, S. Wang, J. Fan, W. Wang, L. Deng, H. Shi, H. Li, Z. Hu, F. Zhang, J. Gao, H. Liu, X. Li, Y. Zhao, K. Yin, X. He, Z. Gao, Y. Wang, B. Yang, R. Jin, I. Stambler, L.W. Lim, H. Su, A. Moskalev, A. Cano, S. Chakrabarti, K.J. Min, G. Ellison-Hughes, C. Caruso, K. Jin, R.C. Zhao, Transplantation of ACE2(-) Mesenchymal Stem Cells Improves the Outcome of Patients with COVID-19 Pneumonia, Aging Dis 11(2) (2020) 216-228.

[5] X. Chen, Y. Shan, Y. Wen, J. Sun, H. Du, Mesenchymal stem cell therapy in severe COVID-19: A retrospective study of short-term treatment efficacy and side effects, J Infect (2020).

[6] Y. Zhang, J. Ding, S. Ren, W. Wang, Y. Yang, S. Li, M. Meng, T. Wu, D. Liu, S. Tian, H. Tian, S. Chen, C. Zhou, Intravenous infusion of human umbilical cord Wharton's jelly-derived mesenchymal stem cells as a potential treatment for patients with COVID-19 pneumonia, Stem Cell Res Ther 11(1) (2020) 207.

[7] M. Hoffmann, H. Kleine-Weber, S. Schroeder, N. Kruger, T. Herrler, S. Erichsen, T.S. Schiergens, G. Herrler, N.H. Wu, A. Nitsche, M.A. Muller, C. Drosten, S. Pohlmann, SARS-

CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor, Cell 181(2) (2020) 271-280 e8.

[8] W. Qu, Z. Wang, J.M. Hare, G. Bu, J.M. Mallea, J.M. Pascual, A.I. Caplan, J. Kurtzberg, A.C. Zubair, E. Kubrova, E. Engelberg-Cook, T. Nayfeh, V.P. Shah, J.C. Hill, M.E. Wolf, L.J. Prokop, M.H. Murad, F.P. Sanfilippo, Cell-based therapy to reduce mortality from COVID-19: Systematic review and meta-analysis of human studies on acute respiratory distress syndrome, Stem Cells Transl Med (2020).